

WHAT IS CLAIMED IS:

1 1. A method for the cryopreservation of sperm comprising:

- 2 (a) obtaining a selected sperm sample;
- 3 (b) cooling said selected sperm sample;
- 4 (c) isolating sperm from said selected sperm sample to produce isolated sperm;
- 5 (d) adding final extender to said isolated sperm to produce a suspension of
- 6 sperm; and
- 7 (e) freezing said suspension of sperm.

1 2. The method of Claim 1 wherein said selected sperm sample comprises a portion of the

2 sperm present in a source sample, said portion of sperm selected for a characteristic, and

3 wherein the sperm concentration in the selected sperm sample is lower than in the source

4 sample.

1 3. The method of Claim 1 wherein said selected sperm sample comprises sex-selected

2 sperm.

1 4. The method of Claim 1 wherein said selected sperm sample comprises mammalian

2 sperm.

1 5. The method of Claim 4 wherein said selected sperm sample comprises bovine sperm.

1 6. The method of Claim 4 wherein said selected sperm sample comprises equine sperm.

1 7. The method of Claim 4 wherein said selected sperm sample comprises porcine sperm.

1 8. The method of Claim 1 wherein said selected sperm sample comprises sperm selected by

2 a method from the group consisting of flow cytometry, a magnetic technique, a columnar

3 technique, a gravimetric technique, a biochemical technique, a technique based on

4 motility of sperm, a technique based on an electrical property of sperm, and any  
5 combination thereof.

1 9. The method of Claim 8 wherein said sperm have been selected by flow cytometry.

1 10. The method of Claim 1 wherein cooling is carried out by reducing the temperature of the  
2 selected sperm sample to about 5°Celsius.

1 11. The method of Claim 10 wherein cooling is carried out over a period of about 60 minutes  
2 to about 240 minutes.

1 12. The method of Claim 1 wherein the final extender added to said selected sperm sample  
2 (each comprise, in addition to a cryoprotectant, one or more of the following components:  
3 a component that maintains osmolality and buffers pH, an organic substance that reduces  
4 cold shock and preserves fertility of sperm, an energy source, a substance that facilitates  
5 sperm capacitation, and an antibiotic.

1 13. The method of Claim 12 wherein said cryoprotectant is selected from the group  
2 consisting of disaccharides, trisaccharides, and any combination thereof.

1 14. The method of Claim 12 wherein said cryoprotectant is selected from the group  
2 consisting of glycerol, dimethyl sulfoxide, ethylene glycol, propylene glycol, and any  
3 combination thereof.

1 15. The method of Claim 12 wherein said component that maintains osmolality and buffers  
2 pH is selected from the group consisting of a buffer comprising a salt, a buffer containing  
3 a carbohydrate, and any combination thereof.

1 16. The method of Claim 12 wherein said component that maintains osmolality and buffers  
2 pH is selected from the group consisting of sodium citrate,  
3 Tris[hydroxymethyl]aminomethane,

4 N-Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid, monosodium glutamate,  
5 milk, HEPES buffered medium, and any combination thereof.

*any one from this claim.*

1 17. The method of Claim 12 wherein said organic substance is selected from the group  
2 consisting of egg yolk, an egg yolk extract, milk, a milk extract, casein, albumin, lecithin,  
3 and any combination thereof.

1 18. The method of Claim 12 wherein said energy source is a monosaccharide selected from  
2 the group consisting of glucose, fructose, mannose, and any combination thereof.

1 19. The method of Claim 12 wherein said antibiotic is selected from the group consisting of  
2 tylosin, gentamicin, lincomycin, linco-spectin, spectinomycin, penicillin, streptomycin,  
3 and any combination thereof.

1 20. The method of Claim 1 wherein, after the addition of the final extender, the sperm sample  
2 and suspension of sperm, respectively, comprise glycerol, sodium citrate,  
3 Tris[hydroxymethyl]aminomethane, egg yolk, fructose, and one or more antibiotics.

1 21. The method of Claim 1 wherein, after the addition of the final extender, said sperm  
2 sample and suspension of sperm, each comprise glycerol, sodium citrate, egg yolk, and  
3 one or more antibiotics.

*See claim 12.*

*these don't say only salt & glycerol is a base.*

1 22. The method of Claim 1 wherein, after the addition of the final extender, said sperm  
2 sample and suspension of sperm, each comprise glycerol, egg yolk, milk, fructose, and  
3 one or more antibiotics.

1 23. The method of Claim 1 wherein said extender has a pH in the range of about 6.5 to about  
2 7.5.

1 24. The method of Claim 1 wherein the sperm are isolated from said selected sperm sample  
2 by centrifugation.

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1 25. The method of Claim 24 wherein said centrifugation allows for at least about 50% to  
2 about 90% recovery of sperm.

1 26. The method of Claim 1 wherein the concentration of sperm in said suspension prior to  
2 freezing is about  $1 \times 10^6/\text{ml}$  to about  $300 \times 10^6/\text{ml}$ .

1 27. A frozen selected sperm sample comprising a portion of the sperm present in a source  
2 sample, said portion of sperm selected for a characteristic.

II 1 28. The frozen selected sperm sample of Claim 27 wherein said frozen selected sperm sample  
2 comprises sex-selected sperm.

1 29. The frozen selected sperm sample of Claim 27 wherein said frozen selected sperm sample  
2 comprises mammalian sperm.

1 30. The frozen selected sperm sample of Claim 29 wherein said frozen selected sperm sample  
2 comprises bovine sperm.

1 31. The frozen selected sperm sample of Claim 29 wherein said frozen selected sperm sample  
2 comprises equine sperm.

1 32. The frozen selected sperm sample of Claim 29 wherein said frozen selected sperm sample  
2 comprises porcine sperm.

1 33. The frozen selected sperm sample of Claim 27 wherein the method used to select said  
2 selected sperm sample comprises a technique from the group consisting of flow  
3 cytometry, a magnetic technique, a columnar technique, a gravimetric technique, a  
4 biochemical technique, a technique based on motility of sperm, a technique based on an  
5 electrical property of sperm, and any combination thereof.

1 34. The frozen selected sperm sample of Claim 33 wherein said frozen selected sperm sample  
2 comprises sperm that have been selected by flow cytometry.

1 35. The frozen selected sperm sample of Claim 27 wherein said frozen selected sperm sample  
2 is produced by a method comprising:

- 3 (a) obtaining a selected sperm sample;  
4 (b) cooling said selected sperm sample;  
5 (c) isolating sperm from said selected sperm sample to produce isolated sperm;  
6 (d) adding final extender to said isolated sperm to produce a suspension of  
7 sperm; and  
8 (e) freezing said suspension of sperm.

1 36. A method comprising using the frozen selected sperm sample of Claim 27 for artificial  
2 insemination or in vitro fertilization.

1 37. The method of Claim 36 comprising using said frozen selected sperm sample for low-  
2 dose artificial insemination.

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A1

add  
B2